Flow Cytometric Diagnosis of Low Grade B-cell Leukemia/Lymphoma

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Flow Cytometric Analysis of Hematolymphoid Neoplasia

- Standard of care-not research
- Guidelines available for medical indications
- Plays a vital role in diagnosis and sub-classification

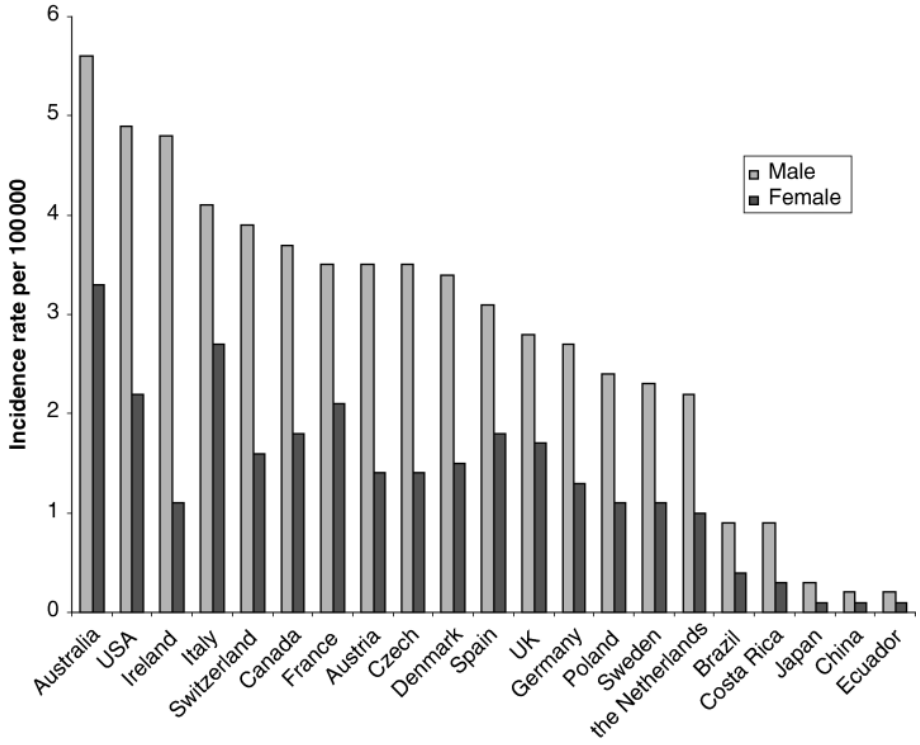
Davis et al.. 2006 Bethesda International Consensus Recommendations on the Flow Cytometric Immunophenotypic Analysis of Hematolymphoid Neoplasia: Medical Indications, Clinical Cytometry. 72B:S5-S13, 2007

Flow Cytometric Diagnosis of Chronic Lymphocytic Leukemia (CLL)

- CLL is the most common leukemia in the western world
- Diagnosis is routinely made by a combination of flow cytometry, WBC and review of clinical history
- Flow cytometric diagnosis is accepted medical practice

Chronic Lymphocytic Leukemia (CLL)

CLL Incidence: by country



Chronic Lymphocytic Leukemia (CLL)

Guidelines for the Diagnosis and Treatment of Chronic Lymphocytic Leukemia: A Report from the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) updating the National Cancer Institute-Working Group (NCI-WG) 1996 guidelines:

The diagnosis of CLL requires the presence of more than or equal to 5x 10^9/L B lymphocytes (5000/µL) in the peripheral blood for the duration of at least 3 months.

Clinical history is relevant

Blood. 2008;111:5446-5456)

Flow Cytometric Diagnosis of CLL :

If detect by flow cytometry the presence of monoclonal B-cells with appropriate immunophenotype in the peripheral blood and at least 5x 10^9/L B lymphocytes (5000/µL)*- Diagnosis is CLL

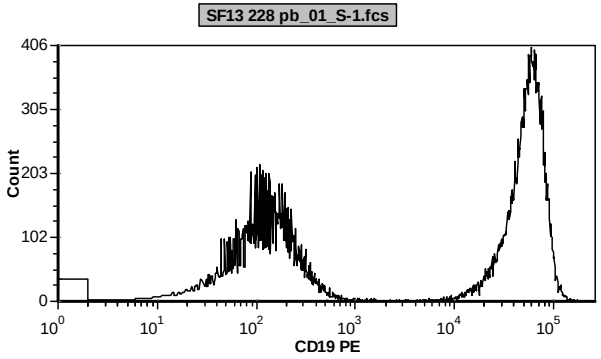
If less than 5x 10^9/L B lymphocytes (5000/µL)- Diagnosis is Monoclonal B-Cell Lymphocytosis

Absolute lymphocyte count elevated: Monoclonal lymphocytosis with clinical lymphocytosis:

Absolute lymphocyte count not elevated: Low count monoclonal lymphocytosis

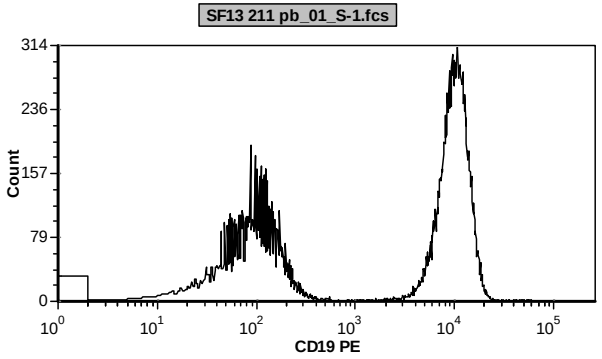
* duration of at least 3 months Clinical history and WBC results relevant

Flow Cytometric Diagnosis of CLL: Not a Chemistry Test But a Clinical Diagnosis



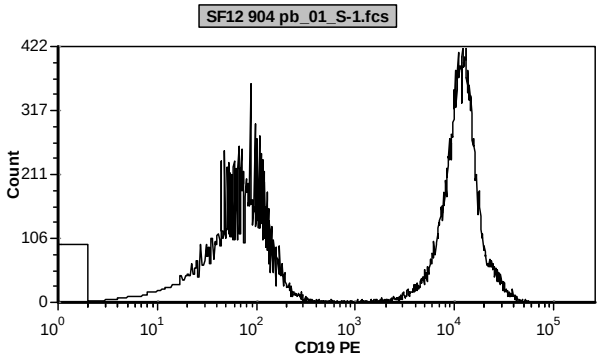
54% B-cells

HCL



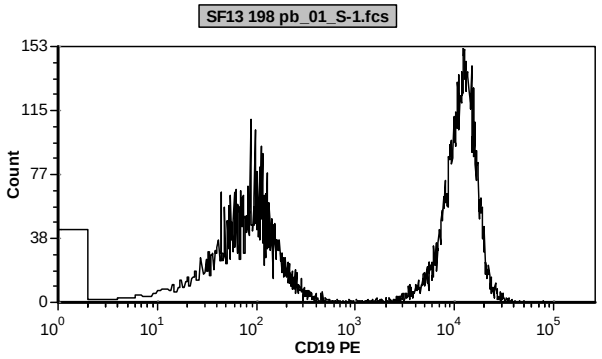
54% B-cells

CLL



48% B-cells

Polyclonal



46% B-cells

Polyclonal

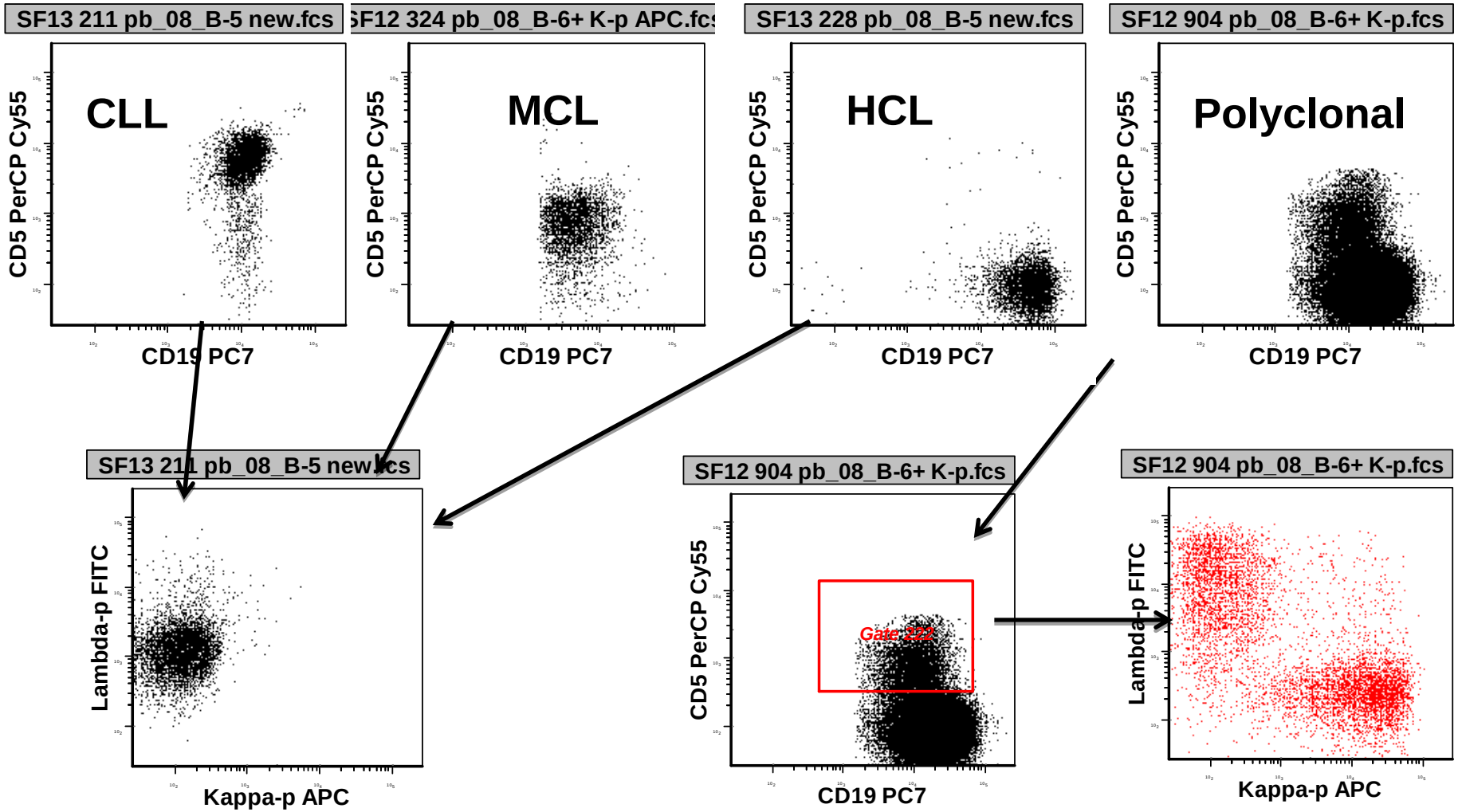
Need more than a B-cell lymphocytosis for Diagnosis of CLL

Differential Diagnosis in CLL:

- Mantel Cell lymphoma
- Splenic Lymphoma with Villous Lymphocytes (SLVL)
- Lymphoplasmacytic lymphoma
- Follicular Lymphoma
- PLL
- HCL
- And if not determining lymphocytosis is B-cell- other non-Hodgkin's lymphomas, including T-cell.

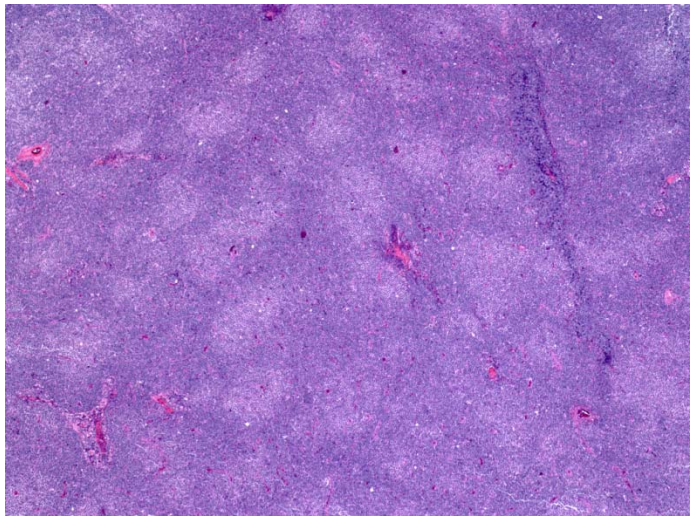
Flow Cytometric Diagnosis of CLL: Not a Chemistry Test But a Clinical Diagnosis

Can we just count CD5+ B-cells?

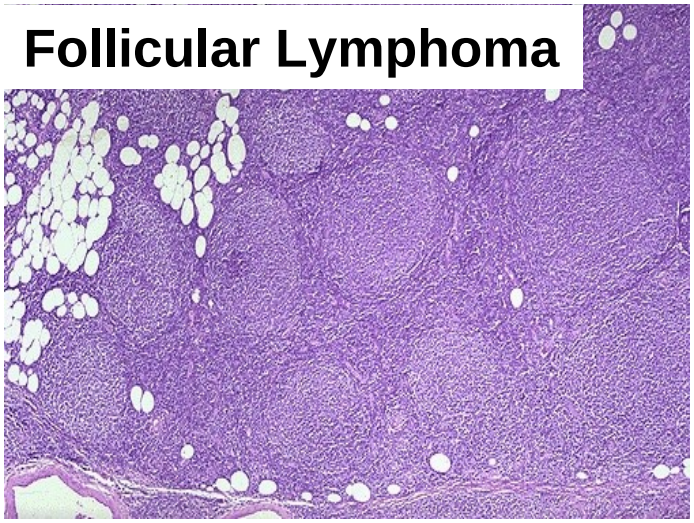


Flow Cytometric Diagnosis of CLL: Similar to Looking at H&E Slide

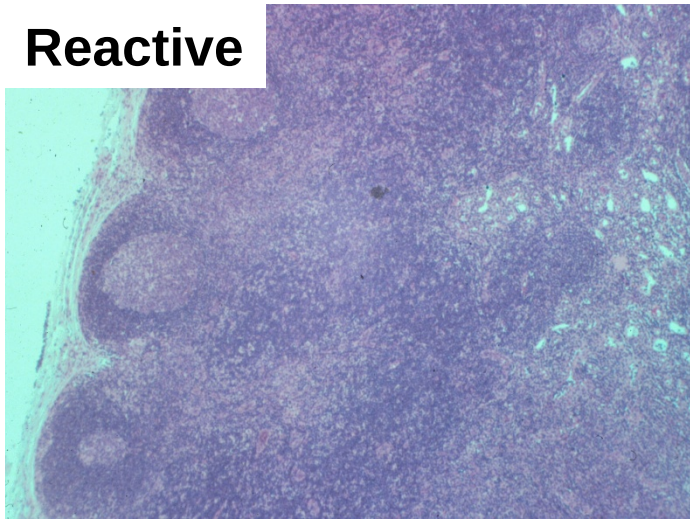
CLL with vaguely nodular growth centers



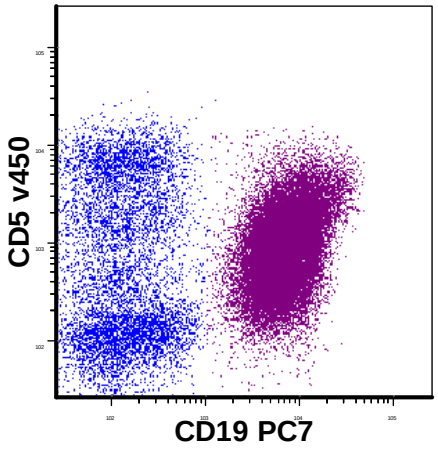
Follicular Lymphoma

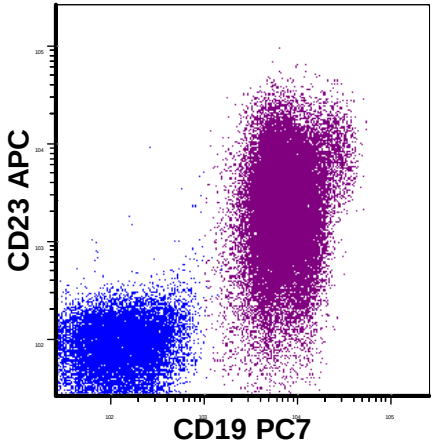


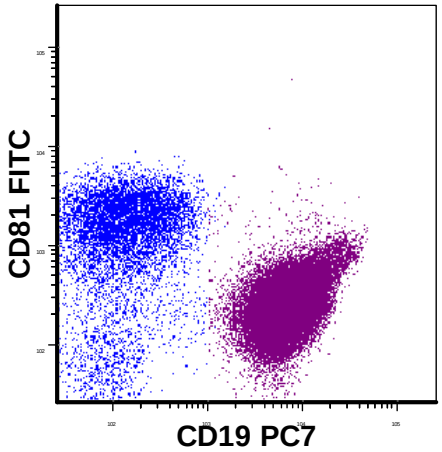
Reactive

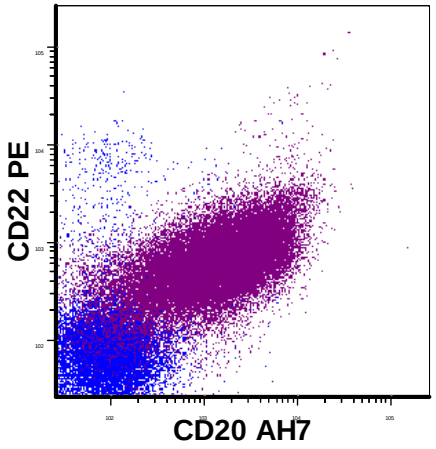


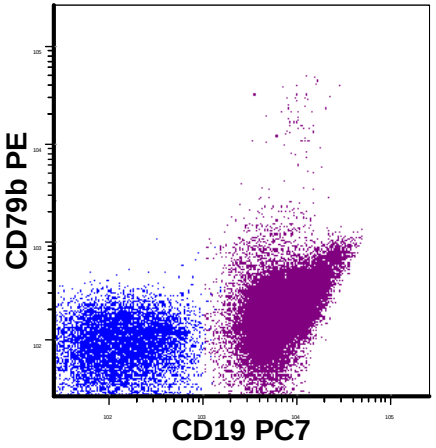
Flow Cytometric Diagnosis of CLL : Appropriate Immunophenotype

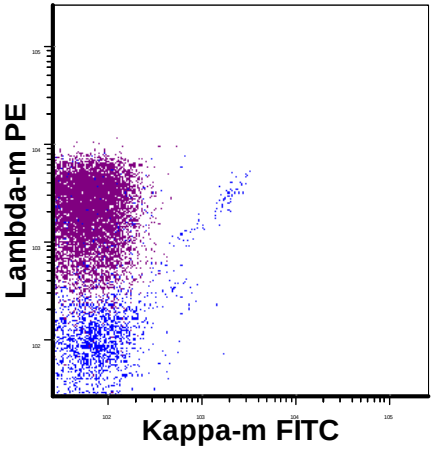












CD19+CD5+

CD23+

Dim to negative CD81

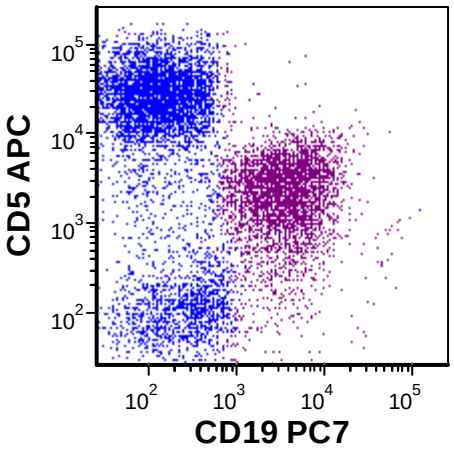
Dim CD20, dim CD22

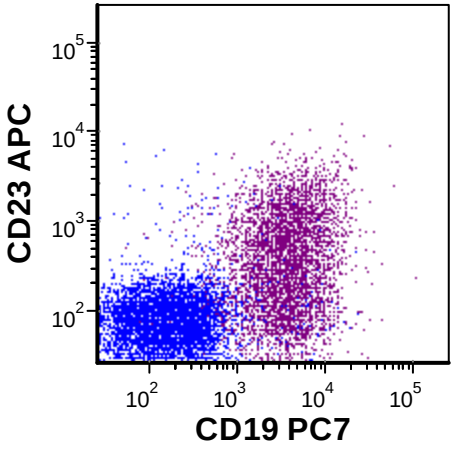
Dim to negative CD79b

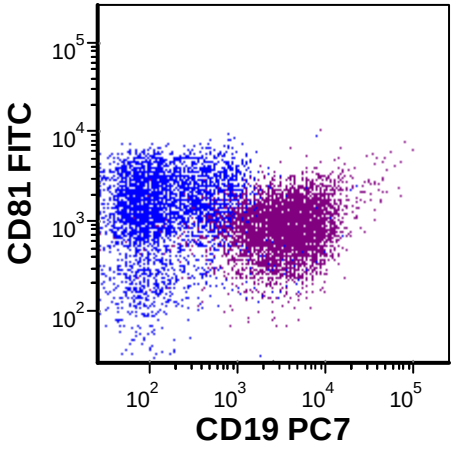
Monoclonal

CLL

Flow Cytometric Diagnosis of CLL : Appropriate Immunophenotype







CD19+CD5+

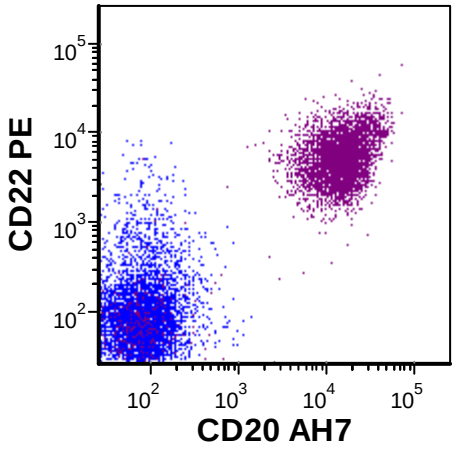
Dim Partial CD23+

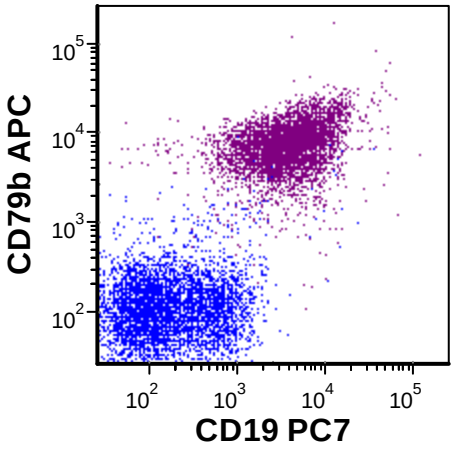
Moderate CD81

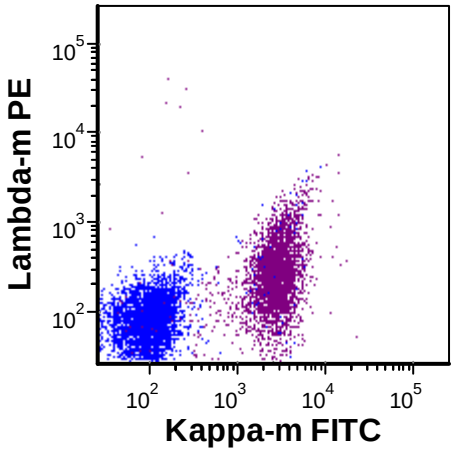
Moderate CD20&CD22

Moderate CD79b

Monoclonal







MCL

CLL Diagnosis is Routinely Performed by Flow Cytometry:

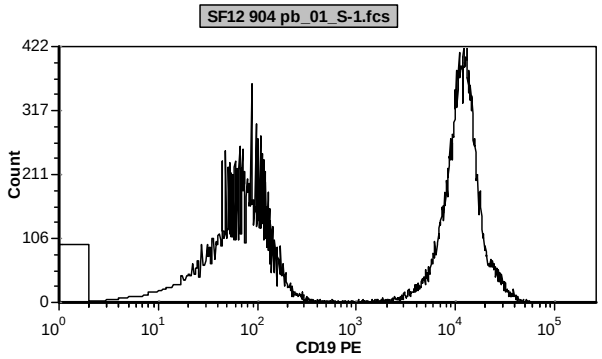
- 5x 10^9/L B-cells (5000/µL)
- At least 3 months duration
- Monoclonal
- Appropriate immunophenotype (CD19+, CD5+, CD23+, dim CD20+, dim CD22+, dim to negative CD79b, dim to negative CD81, CD43+, dim CD45+, dim surface immunoglobulin

Diagnosis made by medical professional based upon identification of abnormal pattern and correlating this with history and other test results (WBC)

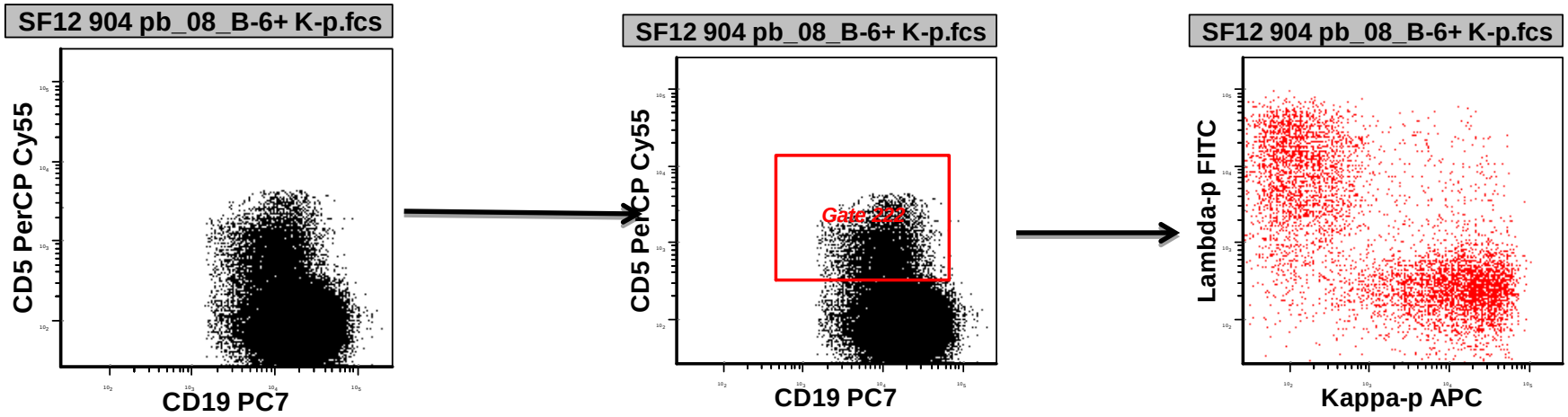
Flow Cytometry Used in Diagnosis and Sub-Classification of B- LPD

- Differentiation of reactive from clonal
- Sub-classification of CD5+ B-cell neoplasms:
  - CLL, MCL, PLL, LCL, HCL, FL,
- Sub classification of CD10+ B-cell neoplasms:
  - FL, Burkitt's. LCL, MCL, HCL
- Sub-classification of B-cell neoplasms with hairy/villous cytoplasmic borders
  - HCL, HCLv, SLVL

Differentiation of reactive from clonal

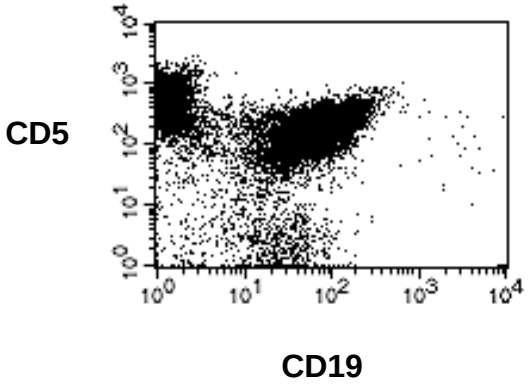


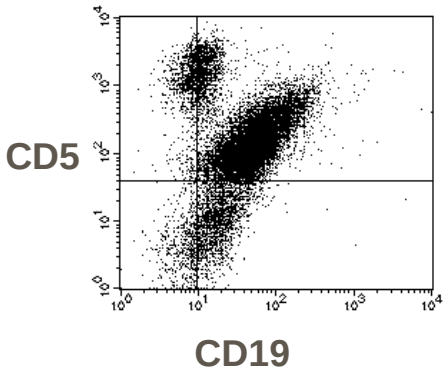
48% B-cells



Subclassification of B- Cell Neoplasms: CD5+

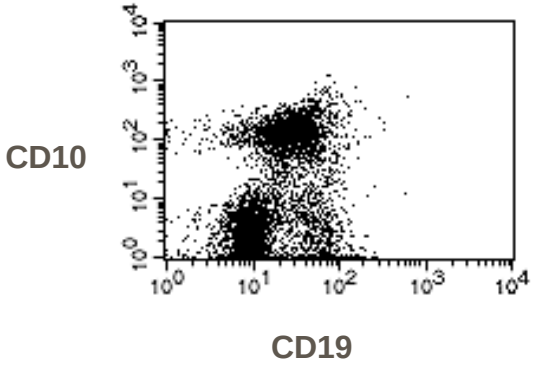
- **MCL:**
  - **+CD5, +CD19, - CD23 (may be dim+),**
  - **moderate CD20, CD22, sIg, CD79b, CD81, CD45,**
- **CLL:**
  - **+CD5, +CD19, +CD23,**
  - **dim CD20, CD22, sIg, CD79b, CD81, CD45**
- **PLL and LCL**

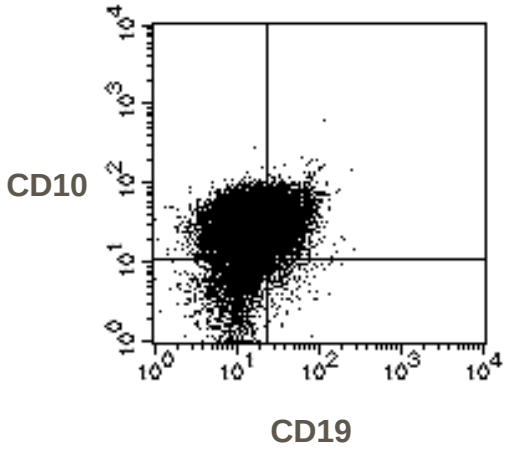




Subclassification of B- Cell Neoplasms: CD10 Positivity

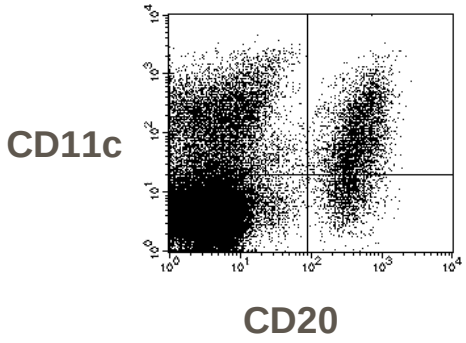
- **FL:**
  - **+ CD10, dim CD19, +CD20, +CD22, +slg, +/-CD23, -CD5, -CD25, -CD11c, -CD103**
- **Burkitt's:**
  - **+CD10, moderate CD19, +CD20, +CD22, +slg, -CD23, -CD5**
- **ALL: Immature**
  - **+CD10, dim CD19, +CD22, +/-CD20, +TdT, -slg, +/-CD34**
- **LCL and MCL**



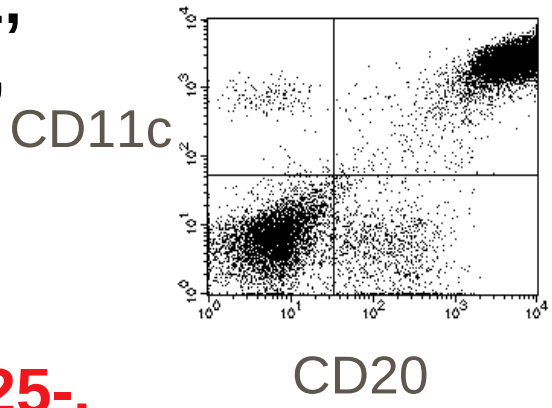


Subclassification of B- Cell Neoplasms: Hairy/Villous Cytoplasmic Borders

- **HCL**
  - **+ CD19, Bright CD20, Bright CD22, Bright CD25, Bright CD11c, CD103, CD123**

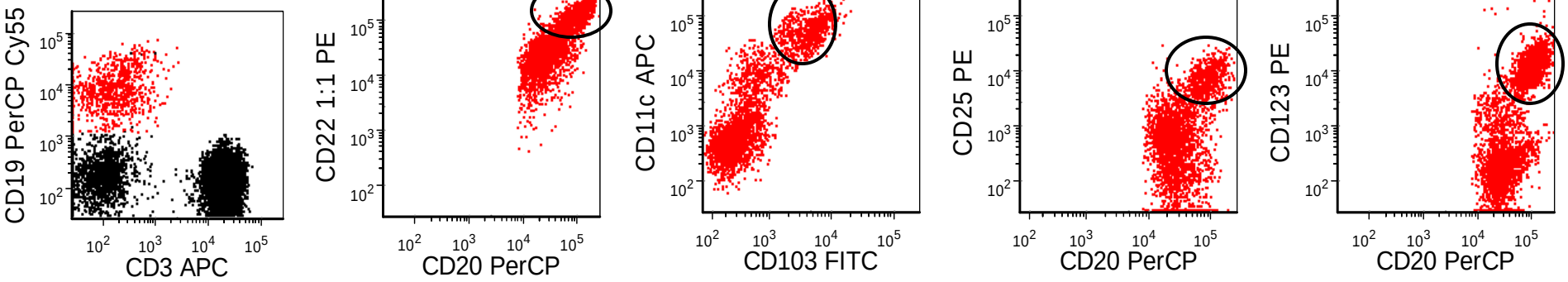


- **HCLv**
  - **+ CD19, Bright CD20, Bright CD22, ++CD11c(can be moderate or dim), +CD103, - CD25, - or dim CD123**

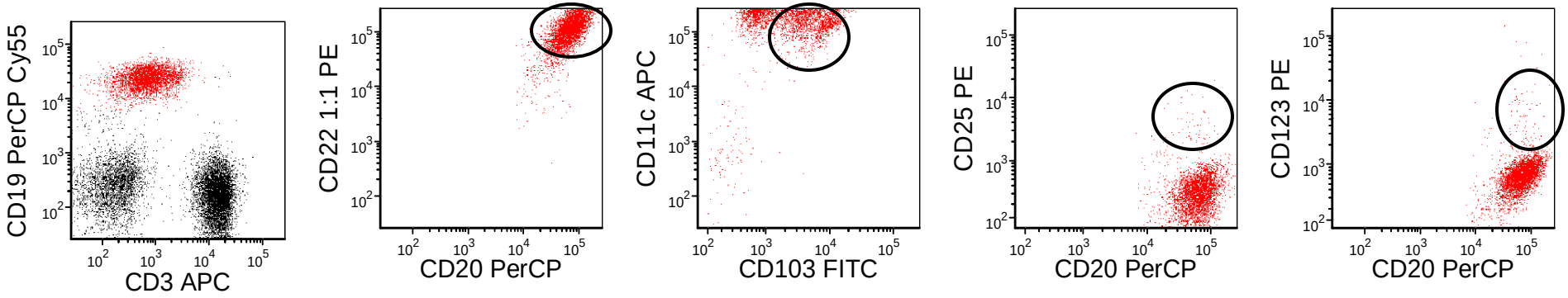


- **SLVL**
  - **+ CD19, Moderate CD20, Moderate CD22, +/- CD11c (dim when +), CD25-, CD103-, CD123-**

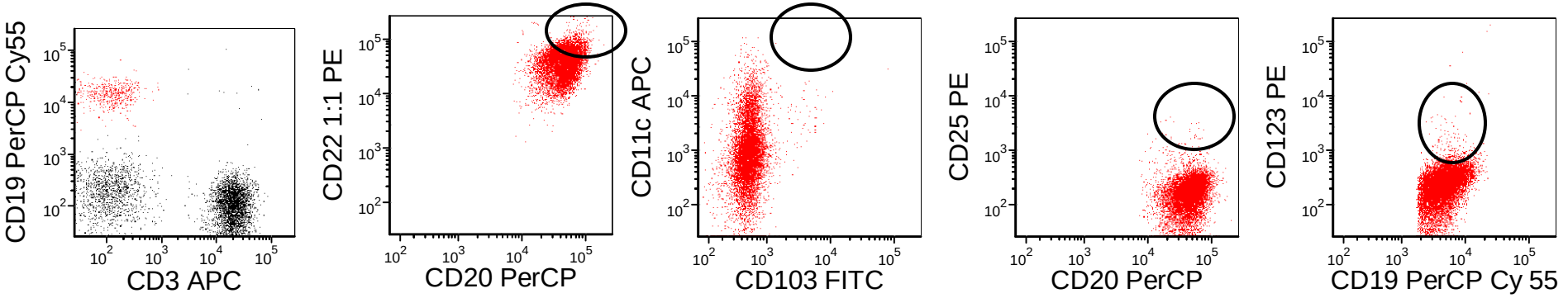
HCL



HCLv



SLVL



Summary

- Flow cytometry is routinely used for diagnosis and sub-classification of B-cell neoplasia
- The diagnosis is made by a medical professional with intimate knowledge of normal immunophenotypic patterns
- Diagnosis is based upon recognition of an abnormal pattern, not a single value, similar to evaluation of morphology.
- Flow cytometric results are correlated with clinical history and the results of other tests for the diagnosis